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<b>(21) International Application Number:</b> PCT/GB93/02167 <b>(22) International Filing Date:</b> 20 October 1993 (20.10.93)  <b>(30) Priority data:</b> 9222021.9 20 October 1992 (20.10.92) GB  <b>(71) Applicant (for all designated States except US):</b> CTS BIOCIDES LTD. [GB/GB]; Cambridge Research Laboratories, 181a Huntingdon Road, Cambridge CB3 0DJ (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> HILL, Martyn, William [GB/GB]; 47 High Street, Hinxton, Saffron Walden, Essex CB10 1QY (GB). SHARMAN, Dennis, Frank [GB/AT]; Dirnbach 56, Post Straden, A-8345 Oststeiermark (AT). RECTON, Peter, Dario [GB/GB]; 16 Hogarth Close, St. Yves, Cambridgeshire PE17 6HJ (GB).	<b>(74) Agent:</b> GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).  <b>(81) Designated States:</b> AU, BB, BG, BR, BY, CA, CZ, FI, GB, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ASSAY FOR CATIONIC SURFACTANTS  <b>(57) Abstract</b>  A cationic surfactant, e.g. a biocide, in an aqueous system is determined by contacting a sample with Direct Yellow or another aromatic dye having sulphonate groups, the dye and the surfactant giving a characteristic colour depending on the concentration of the surfactant, at an appropriate pH, and comparing the concentration at which the characteristic colour is observed with a standard.	

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## ASSAY FOR CATIONIC SURFACTANTS

Field of the Invention

This invention relates to an assay for cationic surfactants.

5 Background of the Invention

Quaternary ammonium pesticides such as Diquat have been determined by HPLC ion-pair systems. A simpler, sensitive assay for quaternary compounds is desirable, especially since cationic surfactants of this type are  
10 widely used, e.g. in waste disposal, shampoos and, as biocides, in cooling towers. Examples of biocidal cationic surfactants that are used in cooling towers, in order to control organisms such as Legionella SPP, are (alkyl) quaternised compounds such as  
15 tetrakis(hydroxymethyl)phosphonium sulphate, tributyltetradecylphosphonium chloride, dimethylbenzylammonium chloride and polymeric biguanide hydrochloride (respectively available under the trade names Tolcide, Bellacide 350, Hyamine 3500 and Vantocil). It is very desirable to  
20 monitor the level of such compounds in water, e.g. from cooling towers and also in cutting fluids, effluents and in the paper industry.

Summary of the Invention

According to the present invention, the presence of a  
25 cationic surfactant in an aqueous sample is determined by contacting the sample with an aromatic dye having sulphonate groups, the dye and the surfactant giving a characteristic colour depending on the detergent concentration, at an appropriate pH, and comparing the  
30 concentration at which the characteristic colour is observed with a standard.

Description of the Invention

The present invention is based on the discovery that, in practical biocide dosage ranges, certain dyes have the  
35 characteristic defined above. Many aromatic dyes, having an appropriately conjugated chromophore, e.g. azo dyes, are known, and dyes of this type suitable for use in the

invention may be determined by simple testing. Examples of dyes that can be used in accordance with the present invention are Direct Yellow 50, Evans Blue and Alphazurine A, having the respective CAS numbers 3214-47-9, 314-13-6 and 3486-30-4. Patent Blue VF may also be used.

The reaction is conducted at a pH at which the interaction occurs. For azo-sulphonate dyes such as Direct Yellow and Evans Blue, in salt form, the pH is usually below 6, more usually below 5, and often much lower, e.g. about 1, for which purpose an acid is added, or a buffer. The pH may also determine colour stability. For Direct Yellow 50 at least, the test may be conducted in 2 M HCl.

In a first embodiment of the invention, a test has been developed to determine Bellacide 350 (e.g. at 0-18  $\mu\text{g/ml}$ ) in water samples. In this test, Direct Yellow 50 is added to a water sample containing Bellacide, and acidified. The resulting colour of the solution is matched to a colour standard representing a given Bellacide concentration.

A similar colour change is given with approximately three times the concentration of Hyamine. Depending on the concentration of the detergent in the sample, dilution may be required in order to give the best match with the standard. Interference between Hyamine and Bellacide is not a practical problem, as only one of the two biocides is part of a dosing regime in any cooling tower system.

The standard is suitably provided in a colour comparator of the type produced by Lovibond, in which different shades are provided on a disc and correspond to a calibrated concentration. The disc is rotated until the one colour showing matches that provided by the adjacent sample.

More specifically, a suitable comparator disc has a range of colours from blue/purple to yellow, respectively corresponding to 0 and 18  $\mu\text{g/ml}$  Bellacide. Shades between the two colour extremes suitably correspond to increments

of 3  $\mu\text{g/ml}$ . Matching may occur immediately after reagent addition.

The test method has been conducted in the presence of various Polymates and the biocides Aquacar, Bronopol, DBNPA, Dithiol, Kathon and MBTC. None of these biocides was found to interfere and only one Polymate exceeded a 5% interference. The test was effective in the presence of 2000 ppm artificial hardness. 60 water samples were spiked with 15  $\mu\text{g/ml}$  Bellacide; satisfactory results were obtained in all cases.

In a second embodiment of the invention, a test has been developed to determine Hyamine 3500 (0-40  $\mu\text{g/ml}$ ) in water samples by adding Evans Blue dye dropwise to a solution buffered to pH 4.6 with sodium acetate-acetic acid solution. A colour change from purple to blue is observed at the end point. The quaternary phosphonium compound Bellacide 350 (and Vantocil) also react, although other biocides or additives used in cooling tower water and that have been tested do not react.

The amount of dye and buffer needed to produce a graduated end-point have been optimised. In addition, a set of standard colour changes has been set up so that an increase in Hyamine 3500 of 5  $\mu\text{g/ml}$  would require an additional one drop of dye to change the colour from purple to blue.

For a dye such as Alphazurine A, an acid dye in RO water, the reaction is usually done at a pH above 10, e.g. about 13, for which purpose a base is added. More particularly, in a third embodiment of the invention, it has been found that Alphazurine A can be used to detect Hyamine 3500 at a concentration range of 0 to 20 ppm; this may be increased to 0 to 40 ppm, by 1:1 dilution, e.g. in increments of 5 ppm. The test comprises only three stages, and can take less than seven minutes to complete. It does not give a single colour but rather a range of colours from turquoise to pink, depending on the concentration of the Hyamine 3500 present.

The end-point of the reaction is a pink colour, but the higher the concentration of Hyamine 3500, the faster this end-point is reached. As a result, assessing the colour obtained after a fixed length of time means that a particular colour can be related to a specific concentration. The colour changes from turquoise to blue to purple to pink.

The amount of sodium hydroxide (as a suitable base) required for the test results in a precipitate in hard water samples. When 5M sodium hydroxide was combined with 0.2M EDTA, this gave satisfactory results in water which had a total hardness of up to 1000 ppm (as  $\text{CaCO}_3$ ).

Addition of 2M hydrochloric acid reduces the pH slightly and this slows down the rate of colour change. It also appears to slightly shift the colour obtained back towards the blue and so generates a wider range of colours.

The following Examples illustrate the invention.

#### Example 1

The following procedure was used:

1. Add 20 ml of 2 M HCl to reaction vessel.
2. Add 3.5 ml of sample to vial containing 86.6  $\mu\text{g}$  freeze-dried Direct Yellow 50, gently swirling the solution around for a few seconds.
3. Immediately pour contents of vial into reaction vessel. Transfer a portion in the reaction vessel back to the vial and then return. Repeat.
4. Transfer contents of reaction vessel to 40 mm cell, placing in right-hand compartment of Lovibond comparator and tap/R.O. water in left-hand compartment and rotate disc until a match is obtained.

#### Example 2

The procedure of Example 1 was followed, except that step 2 was modified as follows:

2. Add 3.5 ml of sample to glass container. Add 25  $\mu\text{l}$  of 0.005 M  $\text{Na}_2\text{S}_2\text{O}_3$  and mix well. Transfer to

vial containing 86.6  $\mu$ g Direct Yellow 50, gently swirling the solution around for a few seconds.

This modification is suitable for use with chlorine interference. The given method will neutralise up to approx. 5 ppm chlorine.

#### Example 3

The following procedure was used:

1. Transfer 20 ml of 2 M HCl to 40 mm Lovibond cell.
2. Add 3.5 ml of a water sample.
- 10 3. Add 65  $\mu$ l of 1.332 mg/ml Direct Yellow 50 (in RO water), mix and compare to disc immediately.

#### Example 4

The following procedure was used:

1. Add 1 ml of sample to a 40 mm Lovibond cell.
- 15 2. Add 5 ml 0.5 M sodium acetate buffer.
3. Fill to the 20 ml mark with R.O. tap water.
4. Add 100  $\mu$ l drops of 56  $\mu$ g/ml Evans Blue. After adding each drop compare to the standard colour expected to be obtained for that number of drops.
- 20 If the sample colour is more purple than the standard, continue adding Evans Blue dropwise, and comparing with the standard colours, until the sample is more blue than the corresponding standard.
- 25 5. The number of drops of Evans Blue needed is directly proportional to the concentration of Hyamine 3500.

#### Example 5

The results shown below were obtained by the following protocol:

To 5 ml sample add 5 ml water followed by 500  $\mu$ l 0.1 mg/ml Alphazurine A. Mix and wait 1 minute.

Add 5 ml of a 5M sodium hydroxide with 0.2M EDTA solution. Mix and wait 3.5 minutes.

35 Add 5 ml 2M hydrochloric acid. Mix and assess the concentration by comparison of the colour with a range of standards.

Results obtained for 0 to 40 ppm Hyamine 3500:

	ppm	
	0	yellowed turquoise
	5	turquoise
5	10	blue - turquoise
	15	blue
	20	purple
	25	purple - pink
	30	pink - purple
10	35	pink + purple
	40	pink

In the presence of other biocides and cooling tower water additives, no major interferences were detected at normal cooling tower dose levels except for Bellacide 350 (see below) and Fenticlor. The procedure has performed well with real water samples, with no unexpectedly high or low results when these water samples have been spiked with Hyamine 3500 in the laboratory.

Example 6

20 Bellacide 350 reacts with about half of the activity of Hyamine 3500. A method for Bellacide 350 has been developed (for which Hyamine 3500 reacts also), following the protocol of Example 1, but waiting 15 minutes after the mixing with NaOH and EDTA, and using 1M HCl.



CLAIMS

1. A method of determining a cationic surfactant in an aqueous sample, which comprises contacting the sample with an aromatic dye having sulphonate groups, the dye and the surfactant giving a characteristic colour depending on the concentration of the surfactant, at an appropriate pH, and comparing the concentration at which the characteristic colour is observed with a standard.
- 5 2. A method according to claim 1, wherein the sample contains up to 40 ppm of the surfactant.
- 10 3. A method according to claim 1 or claim 2, wherein the pH is below 5.
4. A method according to claim 1 or claim 2, wherein the pH is above 10.
- 15 5. A method according to claim 3, wherein the dye is Evans Blue.
6. A method according to claim 3, wherein the dye is Direct Yellow.
7. A method according to claim 4, wherein the dye is Alphazurine A.
- 20 8. A method according to any preceding claim, wherein the surfactant is a quaternised alkylphosphonium or alkylammonium salt.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/02167

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 G01N31/22

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 7918, Derwent Publications Ltd., London, GB; Class D15, AN 79 79-34860B 'Qualitative determinatin of quaternary ammonium surfactants - in turpentine, rosin or waste water using aq. phenyl:azo-naphtol-di:sulphonic acid derivs. as dye' & SU,A,612 165 (FOREST CHEM IND RES) 26 May 1978 see abstract	1-3,8
Y	see abstract --- -/--	1-8

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI  Section Ch, Week 9128,  Derwent Publications Ltd., London, GB;  Class B05, AN 91-206171  PILLIPENKO, A.T. ET AL. 'Quantitative  determination of salts of quaternary  ammonium bases by treating sample with  bromophenol blue in presence of non-ionic  surfactant and photometry'  &amp; SU,A,1 587 427 (KIEV SHEVCHENKO UNIV.)  23 August 1990  see abstract</p>	1-3,8
Y	<p>see abstract</p>	1-8
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A	<p>---</p> <p>WATER RESEARCH  vol. 18, no. 2, 1984, OXFORD GB  pages 223 - 225  VICTORIO T. WEE 'Determination of cationic  surfactants in waste and river waters'  see the whole document</p>	1-8
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02167

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-3992149	16-11-76	NONE	